REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
15-02-2008	THESIS	

4. TITLE AND SUBTITLE
WATER SPARING IN CHRONIC ETHANOL EXPOSURE IS ASSCIATED WITH ELEVATED RENAL RECEPTOR BATA & VASOPRESSIN V2 MRNA IN THE FEMALE RAT

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
MAJ HUCKSTEP ODARO J

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF HAWAII AT MANOA

8. PERFORMING ORGANIZATION REPORT NUMBER
CI08-0006

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
THE DEPARTMENT OF THE AIR FORCE
AFIT/ENEL, BLDG 16
2275 D STREET
WPAFB OH 45433

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Unlimited distribution
In Accordance With AFI 35-205/AFIT Sup 1

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE		31	19b. TELEPHONE NUMBER *(Include area code)*

WATER SPARING IN CHRONIC ETHANOL EXPOSURE IS ASSOCIATED WITH ELEVATED RENAL ESTROGEN RECEPTOR BETA AND VASOPRESSIN V2 RECEPTOR mRNA IN THE FEMALE RAT

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

PHYSIOLOGY

DECEMBER 2007

By
Odaro J. Huckstep

Thesis Committee:

Dr. Catherine Uyehara, Chairperson
Dr. Yu Chong Lin
Dr. Scott Lozanoff
Dr. Jack Somponpun

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Medical and Molecular Physiology.

THESIS COMMITTEE

Chairperson

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract

Fluid handling is known to differ between males and females. Interactions between sex steroids such as estrogen with fluid regulating hormones like vasopressin (VP) are likely key to establishing these differences. Research has identified estrogen receptor (ER) α and β in renal tissue which may affect renal fluid handling. Thus, this study hypothesized that chronic ethanol exposure would elicit different alterations to water load excretion between male and female Sprague Dawley (SD) rats due to changes in renal VP V2 receptor (V2R) or ER mRNA expression. Therefore, in this study we compared 120 minute excretion of a 2% Body Weight (BW) water load between male control (n=6) and ethanol-fed (n=14) rats, and female control (n=26) and ethanol-fed (n=26) rats. Additionally renal papilla mRNA expression of V2R, ERα, and ERβ was compared between male control (n=5) and ethanol-fed (n=15) rats, and female control (n=12) and ethanol-fed (n=17) rats. Female ethanol fed rats showed a 16% reduction in water load excretion ($p<0.05$) compared to controls. RT-PCR analysis revealed that the decreased water excretion in ethanol-fed females was accompanied by a 40% increase in V2R mRNA ($p<0.05$) and a 146% increase in ERβ mRNA ($p<0.05$) in renal papilla tissue compared to controls. In contrast, ethanol treatment in male rats resulted in no difference in water excretion, and yielded no change to V2R or ERβ mRNA expression in the renal papilla. ERα expression was not different between males and females, nor affected by ethanol treatment. Overall, these results suggest that females can better compensate for the dehydrating effects of ethanol exposure by increasing renal

responsiveness to VP via upregulating renal V2R. Also, ethanol specifically upregulates the ERβ subtype in the female kidney which may modulate renal sensitivity to VP.

Introduction

Maintenance of body fluid balance is an essential physiological function that is performed to exacting tolerances by the synergistic action of several regulatory mechanisms (1). The powerful antidiuretic effect of VP in the distal convoluted tubule is critical to maintaining fluid balance as demonstrated by research on the Brattleboro rat (2, 3). Although the primary water sparing, antidiuretic effects of VP are well documented (1, 4), recent research has identified several mechanisms which may alter the production, release, and function of VP (5, 6). Many such alterations display sexual dimorphism (7, 8) and may be linked to female cycle phases (9) and sex hormones. For example, estradiol is believed to diminish the antidiuretic action of VP in female rats (10).

Irregular fluid handling is a hallmark of some of the most widespread diseases today. The Centers for Disease Control and Prevention estimates that one third of US adults has hypertension (11), and also reported that from 1980 to 2005 the number of persons diagnosed with diabetes rose from 5.6 million to 15.8 million (12). Additionally, the prevalence of alcohol abuse is on the rise, most notably among women (13). Now more than ever, we need far better comprehension of sex-based differences in the etiology and progression of these diseases to improve our treatment and prevention capabilities.

VP is both a potent pressor and antidiuretic agent and it is logical to expect that sex differences in VP regulation or action may significantly contribute to sex differences in fluid handling. Furthermore, ethanol is believed to inhibit VP synthesis in the mouse (5), be absorbed and cleared more quickly in females than males (14), and cause long term alterations to fluid handling and sex steroid mechanisms such as reducing VP release and diminishing preovulatory release of luteinizing hormone (5, 15, 16).

Since estradiol has been reported to influence water handling, we performed preliminary research on control female rats which suggested that water load excretion in female rats varies throughout the estrous cycle and peaks at metestrous. Thus, this study attempts to further elucidate the effect of sex and estrous cycle phase on fluid handling and response to ethanol.

The ability to excrete a 2% BW water load was compared between male and female SD rats fed a control or high dose chronic ethanol diet. Water load data were analyzed for variations based on sex and treatment group. Female rat water load data was further analyzed for differences based on ethanol exposure and estrous cycle phase. Renal papilla mRNA was also analyzed for ERα, ERβ, and V2R and compared between male and female and control and ethanol-fed rats.

The research objective of this study was to characterize the effects of high dose chronic ethanol intake on renal fluid handling in both male and female SD rats.

Hypothesis

Chronic ethanol exposure will elicit different alterations to water load excretion between male and female SD rats due to changes in renal V2R or ER expression.

Materials and Methods

Animal Procurement and Quarantine

All procedures followed experimental protocols approved by the Institutional Animal Care and Use Committee. Young adult male and female SD rats were procured from Taconic Farms (Germantown, NY). Upon arrival, all animals were inspected for signs of injury or illness then placed in quarantine for seven days. Rat rooms were kept at 19-22˚C and maintained a 12/12 hour light/dark cycle. Prior to starting liquid diet, all animals were fed ad lib with Purina laboratory rodent blocks and tap water.

Liquid Diet Feeding

At 7-9 weeks of age, all rats were placed on Bio-Serv (San Diego, CA) control (product # F1259SP) or ethanol (product # F1258SP) liquid diet which provided all calorie and water intake. Rats were allowed to feed ad lib up to 3.5ml/day per 10g body weight. To allow ethanol-fed rats to slowly adjust to the full dose of alcohol consumption, ethanol-fed rats received gradually increasing levels of ethanol as follows:

- Three days of 1/3 potency ethanol liquid diet
- Three days of 2/3 potency ethanol liquid diet

- Full potency ethanol liquid diet (ethanol comprises 35% of total calories) provided through harvest

After four weeks on the liquid diet all rats underwent surgery for permanent implantation of catheters.

Surgical Implantation

Bladder cannulation, and catheterization of the femoral vein, femoral artery, and stomach was performed as implemented by Uheyara and Gellai 1993, and Gellai and Valtin 1979 (17, 18) to provide for precise urine collection, IV infusion, collection of arterial blood, and intragastric water load infusion. Rats were anesthetized with isoflurane inhalant during surgery then given diluted acetaminophen elixir (4.5 mg/ml) in water ad lib for 24 hours post-op to mitigate pain. Rats designated for water loading and tissue collection received all surgical implants described above. Rats designated for tissue collection received vascular catheters in the femoral artery and vein.

All surgical implants were regularly maintained to prevent infection and ensure patency and function. Every three days, cannulated bladders were flushed with a neomycin solution made by mixing 1mL of Neosporin G.U. irrigant with 200 mL of sterile water. Vascular lines were flushed with 10% heparinized saline (100 units/mL) then filled with a 50/50 mixture of 1000 unit/mL heparin and 5% dextrose between experiments. If implants could not be made to function adequately on the day of an experiment, the rat was excluded from experimentation for that day.

Animal Training

Individually fitted acrylic restrainers were used during all experiments to safely immobilize the rats and allow access to all implanted lines. Prior to beginning water load experiments, all rats were fitted with restrainers and received 2-3 training sessions in which they were placed in their restrainers for 1-2 hours to acclimatize them to the restrainers.

Female Rat Cycle Phase Determination

The cycle phase of female rats was determined by performing daily vaginal cytology studies on each rat. To collect vaginal smears for analysis, female rats were placed in restrainers, then 10-20 uL of normal saline were carefully pipetted into the vaginal opening, swished 2-3 times, then recovered and transferred to a slide and allowed to air dry. After air drying, the slides were stained according to the following protocol:

- 5 second bath in 95% ethanol, allow to air dry
- 45 second bath in toluidine blue solution (.23g/L)
- Rinse via dip in deionized water
- Air dry

Once stained and dried, slides were analyzed under a light microscope to determine cycle phase as described in previous studies (19).

H20 Load Experimental Protocol

Rats were allowed at least five days of post-op recovery time prior to water load experiments. Water load experiments were completed after a total of 5-6 weeks on the liquid diet. Depending on the condition of the rat and indwelling catheters, between one and three water loads were performed on each rat, this allowed for optimal use of experimental animals and water load data collection from multiple phases of the estrous cycle for female rats.

To ensure measurement of chronic rather than acute effects of ethanol on water handling, ethanol group rats were placed on control diet the night before undergoing water load experiments to ensure significant levels of ethanol were not present in their system during the experiment.

On the day of the experiment, rats were placed in their restrainers and allowed to equilibrate for 60-90 minutes while receiving a baseline IV infusion of normal saline at the rate of 10ul/min per 100g body weight. This baseline infusion was sustained through the experiment to maintain euhydration up to H20 loading and assured that changes in urine flow and fluid chemistry were attributable to H20 loading dynamics.

Throughout the experiment, heart rate and blood pressure were monitored by a pressure transducer connected to the arterial catheter and timed urine collections were taken prior and subsequent to water load administration. Baseline urine production rates were calculated from pre-H20 load urine collections. After the equilibration period, baseline urine collections were made, then a 2% body weight water bolus was administered over two minutes via the IG catheter. Baseline urine production values

were deducted from post H20 load urine production values to determine the volume excreted in each ten minute collection period.

Plasma samples were collected from each rat prior to the water load (P_0), 10 min after the water load (P_1), and 130 min after the water load (P_2). For plasma sampling, whole blood was collected from the arterial catheter then centrifuged for approximately 1 minute, the separated plasma was then collected with a pipette. The blood cells were reconstituted to the original whole blood volume with 10% heparinized saline solution (100 unit/mL) then returned to the rat via the arterial catheter. Capillary tube hematocrit samples were taken concurrently with blood draws for all plasma samples.

All urine samples were collected in pre-weighed vials and urine volumes were determined gravimetrically using an assumed specific gravity of 1. Two 15 minute urine collections (U1 & U2) were collected after the equilibration period but before the water load. U1 and U2 were used to calculate nominal baseline urine production rates. U3 through U15 were ten minute urine collections with U3 starting immediately prior to administering the water load. All urine and plasma samples were measured for osmolality, Na (mmol/L), and K (mmol/L). Osmolality was measured with both the Fiske Micro-Osmometer Model 210 and the Advanced Instruments Micro-Osmometer Model 3MO Plus. Both osmometers utilize the freezing method (20) to determine sample osmolality. The Medica Easy Electrolytes analytical unit was used to measure NA and K in plasma samples, urine Na and K were measured with the Beckman CX3 Synchron Clinical System.

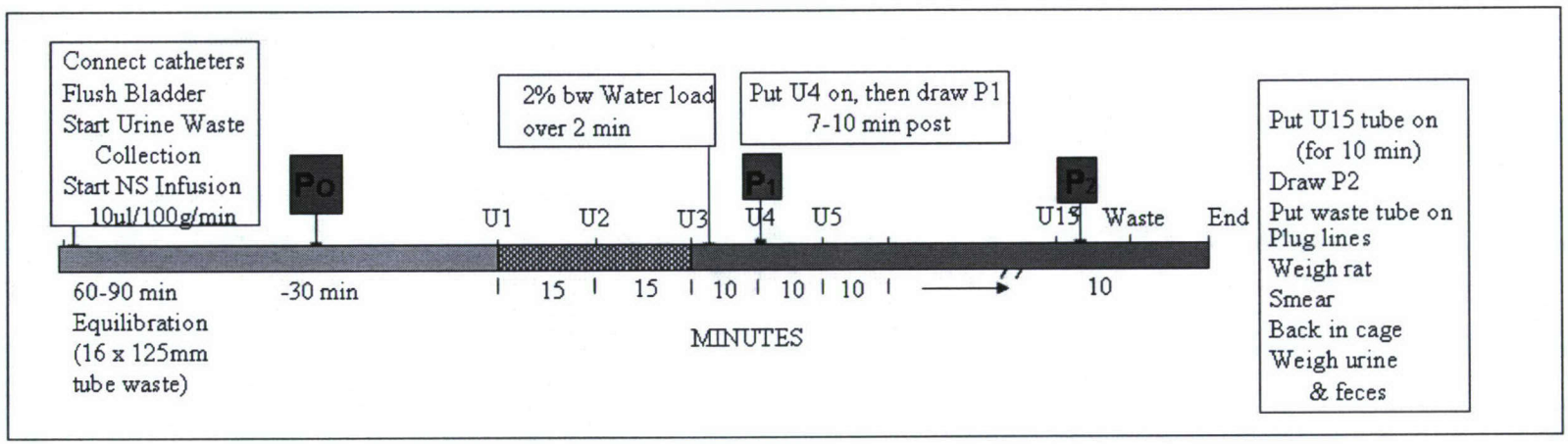

fig. 1 Protocol Time Line For Determination of Water Load Excretion

Water Load Excretion Calculations

Water load analysis was based on data from successful experiments in which the rat was deemed healthy and nominally hydrated at the start of the experiment, indwelling catheters performed properly, no ethanol was detectable in the blood, and no clinical sequelae were observed in the kidneys upon harvest.

Water load excretion values were based on the percentage of the of the water load that was excreted by the rat in the 120 minute period following administration of the water load. The excreted volume was calculated by subtracting the baseline urine production (rate calculated from U1 and U2) from the volume of urine excreted and collected in urine samples U3-U14 (fig 1). This ensured nominal baseline urine production was not counted toward H20 load excretion calculations. This calculation is summarized by the following equation:

$$V_e = 100*[(V_E - V_B)/V_{laod}]$$

V_E = total volume of urine collected in 120 minutes following water load

V_B = volume of urine produced in 120 minutes at baseline production rate

V_{laod} = volume of H20 load administered (2% of rat body weight)

V_e = urine volume excreted as a percentage of the actual water load administered.

Tissue collection

Rats were euthanized one at a time by administering 1ml of pentabarbitol into the venous catheter. Each kidney papilla was immediately dissected out, placed in RNA Later then stored at -70°C until RNA extraction was performed. Rats were allowed at least 18 hours to stabilize from experiments before tissue harvest. No ethanol was detected in the blood and no abnormalities or other clinical sequelae were observed upon harvest.

mRNA Extraction and RT-PCR Analysis

A square section of ~2.5mm^2 was excised from the medial margin of the papilla, then total RNA was extracted with Ambion's RNAqueous 4 PCR kit. The optional DNA digestion step was performed on all samples to remove any trace genomic DNA. For each sample, 4 uL of total RNA sample was reverse transcribed using the Biorad iScript cDNA kit and the Perkin Elmer GeneAmp 9600 thermocycler.

RT-PCR was then performed on cDNA samples using either the Bio-Rad I-cycler or IQ5 I-cycler thermocycler for RT-PCR. Specific primer pairs and hybridization probes were used for each gene (ß-actin, ERα, ERß, and V2R, Fig 2), all primer pairs are intron-spanning. RT-PCR plates were run with ß-actin as a reference against a target gene, and each plate contained samples from multiple study groups. The following thermal cycling profile was used for all gene assays:

1. 3 minutes at 95 degrees C
2. *30 seconds at 95 degrees C

3. *1 minute at 62 degrees C

4. 4 degree C hold

*Steps 2 and 3 performed 45 times

Gene	Fwd Primer 5' to 3'	Rev Primer 5' to 3'	Probe (fam)
V2R	ggatgacactggtgattgtg	cagcagcatgagcaacac	tgggatccggaagctcctctgg
ERα	gacataatgactacatgtgtc	gtatcccgcctttcatcatg	cagctacaaaccaatgcaccatcgata
ERß	ggagtatctctgtgtgaagg	cctggtttgcagaagccaag	catgatcctcctcaactccagtatgtac
ß-actin	caatgagctgcgtgtgg	caacacagcctggatgg	ctcggtgagcagcacaggggtg

fig 2. Primer and Probe Sequences for PCR Analysis of V2R, ERα, and ERß, mRNA Expression

Optical data files from each RT-PCR plate were analyzed using the Biorad IQ5 software package to determine Ct values for each sample. To further normalize plate to plate comparability, a consistent threshold value was maintained across all experimental plates for each particular gene. For ß-actin, ERα, and V2R, a threshold value of 500 was selected for all Ct measurements, a value of 150 was selected for all ERß measurements. All gene expression levels were normalized relative to ß-actin and calculated using the $2^{-\Delta\Delta Ct}$ method (21, 22). Expression levels were calculated using assigned rather than actual copy number values, yielding scaled expression values that provided comparative consistency between multiple plates and samples, but not actual copy number counts for each sample. Individual gene expression levels were compared between study groups. Plate to plate consistency was verified by calculating the coefficient of variability (Cv) in addition to the s.e.m. of a control which was run on each plate for a given gene (Table 2).

Table 1
Variability of Ct Values of Control Samples Across Experimental PCR Plates.

GENE	Ct	Cv	n
V2R	25.7±.17	1.2%	6
ERα	32.6±.23	1.7%	6
ERß	35.0±.30	2.1%	6
ß-actin	25.7±.17	2.4%	18

(values = mean ± s.e.m.; Cv= coefficient of variability)

Data Analysis

Results are expressed as means ± the standard error of the mean. Two way ANOVA was used to compare differences between groups and sexes as well as between estrous cycle phases in females with post hoc comparisons using a Tukey-Kramer's test. A p-value of less then 0.05 was considered statistically significant.

Results

Water Load Excretion Protocol

Males

Ve between male control (82±5, n=6) and ethanol rats (79±5, n=14) was not different (T-test fig 3).

Females

Ve in control (114±5, n=26) and ethanol-fed (98±4, n=26) female rats was significantly higher than corresponding than male rats (T-test, $p<0.05$, Fig. 3). Overall, Ve of ethanol-fed females was lower than Ve of control females (T-test, $p<0.05$, fig 4). When females were compared by estrous cycle phase, Ve tended to be lower in ethanol-fed females compared to control females in every phase except metestrous although this trend did not achieve statistical significance (fig 4).

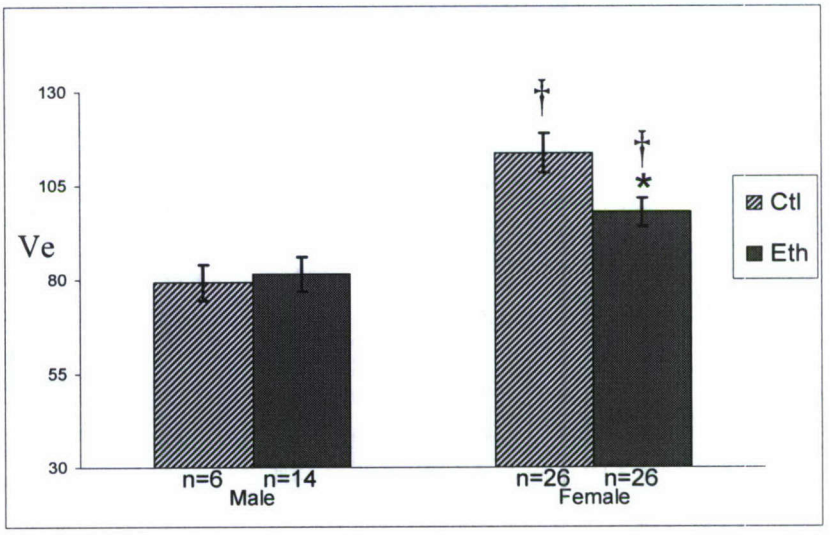

fig 3. Effect of Chronic Ethanol Exposure on Water Load Excretion in Male & Female Rats. Water load excretion did not differ between male control and male ethanol-fed rats. In contrast, female ethanol-fed rats exhibited significantly lower water load excretion than female control rats indicating water sparing in female ethanol-fed rats. (values = mean ± s.e.m. * = significantly different from control female rats, Tukey's Test $p<.05$; † indicates significantly different from corresponding male rats, Tukey's Test $p<.05$)

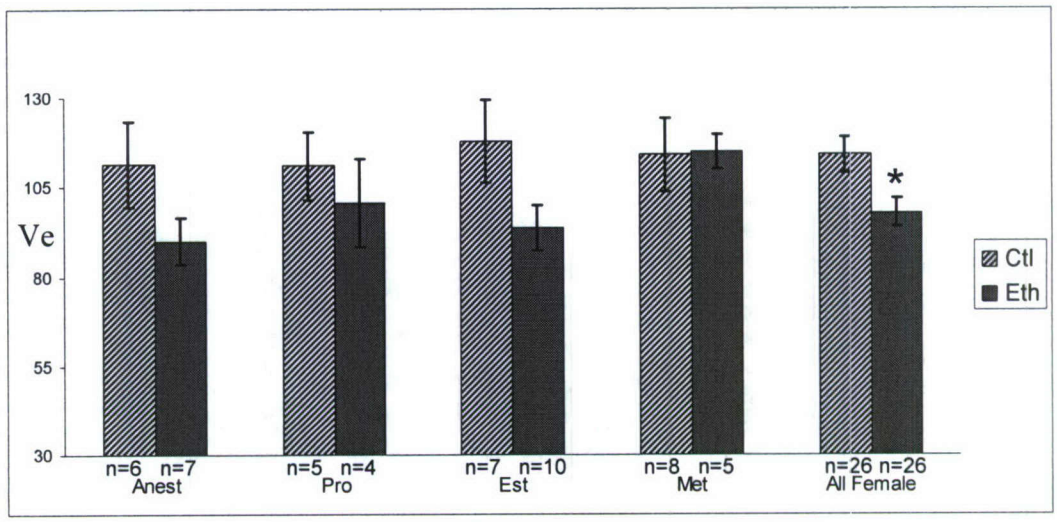

fig 4. Effect of Chronic Ethanol Exposure on Water Load Excretion at Different Estrous Cycle Phases in Female Rats. Ve tended to be lower in ethanol-fed females than control females in every phase except metestrous though this trend did not achieve statistical significance in any single phase. Overall, ethanol-fed female Ve was significantly lower than control females showing significant water sparing in ethanol-fed females. (values = mean ± s.e.m. *= significantly different from control female rats, Tukey's Test $p<.05$)

Osmolality and Hematocrit

Basic analysis of plasma and urine osmolality before water load (baseline), 10 minutes after water load, and 120 minutes after water load revealed the expected drop in urine and plasma osmolality following water loading. Plasma osmolality (POsm) measured 10 minutes after water load administration was significantly lower than baseline POsm (measured 30 min prior to water administration) in all four study groups indicating absorption of water load within 10 minutes. POsm measured 120 minutes after water load administration was significantly lower than baseline only in male ethanol-fed rats (fig 2, Student's T-test, $p<.05$), indicative of a degree of water load retention

combined with a slightly increased baseline POsm. All other groups returned to baseline levels (fig 2).

Urine osmolality (UOsm) measured 10 minutes after water load administration was significantly lower than baseline UOsm (measured 10 min prior to water administration) in all four study groups, indicating absorption of water load within 10 minutes (fig 2, Student's T-test, $p<.05$). Uosm measured 120 minutes after water load administration was significantly different from baseline in only female ethanol-fed rats, indicative of a degree of residual diuresis in ethanol-fed females, but a return to baseline in all other groups (fig 2, Student's T-test, $p<.05$). Baseline UOsm in ethanol-fed male and female rats was significantly higher than baseline Uosm in corresponding control rats (fig 2, Student's t-test, $p<.05$).

Table 2
POsm, UOsm, & Hct Before & After Water Load in Male & Female Control & Ethanol-fed Rats

	Males		Females	
	Control n=6	Ethanol n=14	Control n=25	Ethanol n=26
POsm				
before H_2O load (baseline)	293±1.6	296±1.0	295±0.9	295±1.2
10 min after H_2O load	288±1.5 *	289±1.2 *	290±1.1 *	289±1.3 *
120 min after H_2O load	291±1.5	292±1.1 *	294±0.9	293±1.0
UOsm				
before H_2O load (baseline)	474±68	698±75 †	441±28	593±53 †
10 min after H_2O load	99±13 *	86±6 *	81±7 *	104±7 *
120 min after H_2O load	471±33	576±50	429±19	487±168 * ‡
Hct				
before H_2O load (baseline)	40.3±1.5	39.7±0.7	38.8±0.5	38.9±.0.6
10 min after H_2O load	39.8±1.2	40.5±0.6	40.0±0.5	39.2±0.6
120 min after H_2O load	39.5±1.3	39.4±0.6	39.3±0.5	39.2±0.6

. In all groups, water load resulted in a significant drop in POsm and UOsm within 10 minutes of water loading.
(values = mean ± s.e.m.
* = significantly different from baseline, Student's t-test $p<.05$;
† = significantly different from control-fed, Student's t-test $p<.05$;
‡=significantly different from male ethanol-fed, Student's t-test $p<.05$)

Renal Papilla mRNA Expression of ERα, Erβ, and V2R

Single Gene Expression Levels

No significant mRNA expression differences were identified between male control and male ethanol-fed rats for any genes measured. Likewise, female control and ethanol rats showed no significant difference in their expression of ERα mRNA (fig 5). There was however a statistically significant 146% increase in ERβ mRNA expression in ethanol-fed females (0.63±0.14, n=17) with respect to control (0.26±.04, n=12) females (T-test, $p<0.05$, fig. 6). There was also a mean 40% increase inV2R mRNA expression in

female ethanol-fed rats (0.88±0.11) with respect to female control rats (0.63±0.04) (T-test, $p<0.05$, fig. 7).

Gene expression comparisons across male and female groups revealed the following findings. ERα mRNA expression was not different between any two groups. ERβ mRNA expression was lower in control female rats (0.258±.039, n=12) than male rats (0.588±.109, n=20 fig. 6). V2R mRNA levels were significantly higher in ethanol-fed females (0.877±0.107) than ethanol-fed males (0.600±0.060) (T-test, $p < .05$, fig 7)

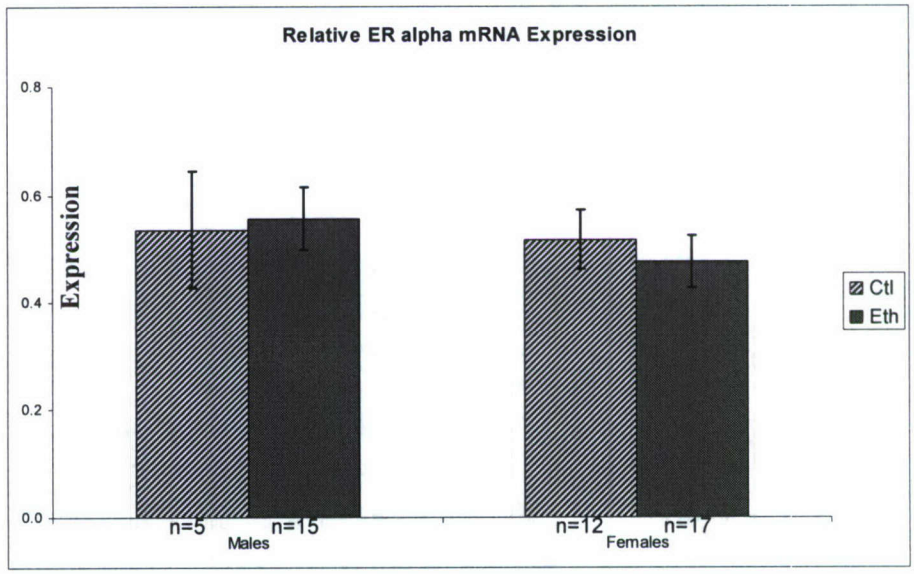

fig 5. Relative ERα Expression in Renal Papilla Tissue in Male & Female Rats
RT-PCR analysis of renal papilla tissue revealed no significant differences between study groups. (values = mean ± s.e.m)

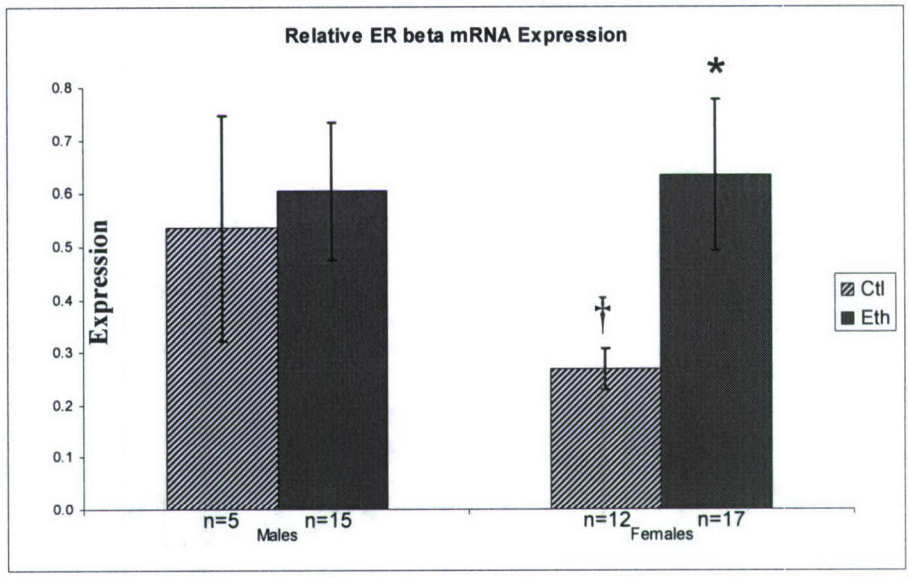

fig 6. Relative ERβ Expression in Renal Papilla Tissue in Male & Female Rats
RT-PCR analysis of renal papilla tissue revealed no significant differences between control and ethanol-fed male rats. Mean ERβ expression in ethanol-fed females was significantly higher than female controls, additionally, control females expressed significantly less ERβ mRNA than male controls. (values = mean ± s.e.m. * = significantly different from control female rats, Tukey's Test $p<.05$; † = significantly different from control male rats, Tukey's Test $p<.05$)

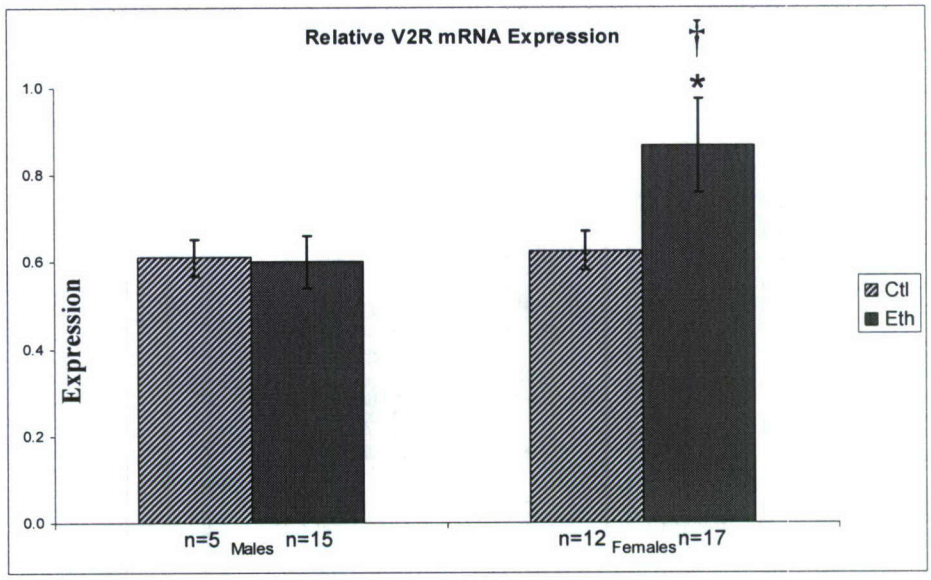

fig 7. Relative V2R Expression in Renal Papilla Tissue in Male & Female Rats
RT-PCR analysis of renal papilla tissue revealed no significant differences between control and ethanol-fed male rats. Mean V2R expression in ethanol-fed females was significantly higher than control females and ethanol-fed males. (values = mean ± s.e.m. * = significantly different from control female rats, Tukey's Test $p<.05$; † = significantly different from corresponding male rats, Tukey's Test $p<.05$)

Single Gene Expression Levels
***note, because expression levels are scaled individually for each gene, these charts do not allow for expression of one gene to be directly compared against another gene*

Discussion

Overview

This study demonstrates that chronic exposure to a high dose of ethanol triggered a compensating ability of female SD rats to hold on to water whereas the same exposure to ethanol in male SD rats produced no change in water load handling. The reduced water load excretion in female ethanol rats was also accompanied with increased V2R and ERβ mRNA expression in the renal papilla, but mRNA levels in male ethanol-fed

rats were not significantly different from male controls. These data help to define yet another thread in the broad canvas of mechanisms for gender distinctions in fluid handling.

Accurately interpreting the effects of sex hormones on fluid balance poses a unique challenge because their actions are manifold and often opposing. Wang, Crofton, Brooks and Share (10) demonstrated that estradiol can attenuate the antidiuretic effect of VP in female rats under control conditions. Characterizing interactions between sex specific effects is also complicated by female reproductive cycling which has been shown to affect water load handling (23), alter renin concentration and activity (24) modulate responsiveness to VP (25), and alter plasma volume and hematocrit (9, 26, 27).

Hematocrit and Osmolality

The baseline UOsm of male and female ethanol-fed rats was significantly higher than control male and female rats. Female ethanol-fed rats tended to have slightly lower POsm than control females in contrast to male ethanol fed rats which tended to have slightly elevated POsm compared to control males. This is consistent with the dehydrating effects of ethanol, in the case of female ethanol-fed rats, there was adequate compensation via increased renal V2R to maintain basal POsm.

In contrast to ethanol-fed females, male ethanol-fed rats tended to have slightly higher plasma osmolality than male controls, indicating an overall loss of water and shift of the POsm set point associated with alcohol intake. This further supports to the concept

that female rats are able to compensate for ethanol exposure with a renal fluid handling change by retaining water whereas male rats do not. Analysis of hematocrit revealed no significant trends indicating equilibration of fluid balance amongst fluid compartments.

Water Excretion and V2R

Previous research in our lab indicated that male rats fed a low dose (5% of total calories from ethanol) chronic ethanol diet were able to compensate via a modest reduction in water load excretion by up-regulating renal V2R. However, the high dose of ethanol provided in this study apparently abolished the capacity for the male rat to compensate by retaining water. It is likely that the adaptations previously observed in males with low ethanol dosing were overwhelmed by the substantially higher dose of alcohol in this study.

The greater Ve of all female rats compared to male rats indicates that the overall effect of estrogen on water sparing mechanisms is probably inhibitory under normal physiological conditions. The reduced water load excretion of ethanol-fed females compared to control females probably results from V2R upregulation which magnifies the antidiuretic effect of vasoporessin in the kidney and helps the animal to conserve water.

Overall, female rats displayed a greater mean Ve than males. The group of all females combined, and the group of all control females exhibited a slight "overshoot" of 100% water load excretion of 5.8% and 13.8% respectively. Our previous studies suggest that this is probably due to a nominal level of suppressed VP response in females.

As a whole, female rats in this study were clearly more efficient at excreting a water load than male rats, even when the females' V_e was diminished by chronic ethanol treatment. Overall, the changes observed in the ethanol females may be indicative of a female specific compensatory capability that helps mitigate the generally dehydrating effects of ethanol exposure.

Vc and Cycle Phase

Though no significant difference was detected between ethanol and control females in any single cycle phase, additional studies with more subjects may show significance in the strong trend of lower mean Ve for ethanol-fed females in every phase other than metestrous. Additionally, since ER expression is thought to be modulated by estrogen levels (28) it is likely that ER levels in the female kidney could vary throughout the estrous cycle and cause a fluctuation in renal responsiveness to estradiol throughout the estrous cycle. Further measurements of estrogen levels and ER levels at different estrous cycle phases are warranted.

Morphological Component of Sex Differences in Renal Function

We observed that control female rats excreted more water than male rats. It is possible that without any difference in hormone levels between males and females that a difference in water excretion capacity exists simply due to differences in anatomical kidney size. Sex differences in kidney morphology contribute to an overall sex difference in renal fluid handling. MRI measurements show that the average male kidney

has one third greater volume than the average female kidney (29). This increased kidney size allows for more long intermedullary nephrons which are optimized for water sparing. Additionally, the increased renal volume in males raises the filtrate dwell time in the kidney which allows more opportunity for transport mechanisms to reclaim water from the nephron. This morphological dichotomy establishes underlying differences in male and female kidney function which combine with sex specific endocrine dynamics that produce a baseline sex difference in renal handling. In summary, these differences result in the female kidney being slightly tuned toward water excretion in contrast to the male kidney which is slightly tuned toward water retention. This is consistent with the water excretion differences observed in control rats in this study.

Estrogen Receptors in the Kidney

Ongoing research continues to add valuable knowledge to this field, but there are still significant gaps including the specific actions of the ER in the kidney. Sun et all (30) reported that female rats displayed greater compensatory kidney growth following uninephrectomy than males, but ERα knockout females showed the same level of compensatory kidney growth as males. This suggests that the primary role of ERα in the kidney may not be related to water handling. In the Taylor and Al-Azzawi (31) study on ER in the human kidney, they identified ERα in the interstitial cells of the kidney, and ERβ in the renal collecting ducts.

In the brain, the actions of ERβ primarily modulate endocrine functions (32). If ERβ performs a similar role in the kidney as well, it's localization in the collecting duct

(31) makes it a potential candidate for involvement in locally altering the action of VP in the renal collecting duct. If that is the case, up-regulation of ERβ in the kidney could either support or oppose the antidiuretic effect of V2R up-regulation in the same region.

ERβ is generally recognized as a nuclear transcription factor (33), but there is also evidence for non-nuclear localization of ERβ (33, 34). One potential mechanism by which ERβ could alter renal response to VP is by changing the transcription rate of aquaporin genes. Aquaporins are rapidly produced in response to VP in the kidney and are directly responsible for allowing water to move out of the nephron and thus be conserved by the body, as a result, altering aquaporin transcription rates would be a highly effective means of mediating renal response to VP. Alternatively, since V2R functions through a stimulatory G protein mechanism, ERβ could potentially alter response to VP by affecting signal transduction pathways by protein-protein interactions (35) which could ultimately impact transduction and the production or degradation rate of second messengers. Another possibility allowing for ERβ V2R interaction is the influence ERβ may have in the recycling of V2R themselves. Recently, it has been shown that V2R surface receptors are continuously moving from the cell surface through endocytosis into the cytoplasm and next to the nuclear ERβ receptor (36).

Interpretation of ERα Results

Since there was no observed change in ERα mRNA levels in this study, it appears less likely that ERα is heavily involved in fluid handling in the kidney. This is supported by the observation that ERα mRNAs were stable across all groups in the study.

However, if ERα is involved in fluid handling, its expression in the interstitial cells (31) suggests that its role could be in mediating mechanisms such as urea transport in the interstitium rather than having a specific transmembrane tubular effect on water handling.

Interestingly, bivariate analysis revealed a significant correlation between V2R and ERα exclusively in male ethanol-fed rats ($p<0.05$, $r=.56$). It is unclear if this represents any meaningful physiological action or not. It is possible that ERα and V2R may have a unique direct or indirect regulatory influence on each other in the male alcohol-fed rat. Another possibility is that a third factor exerts a similar effect on the expression of both genes. At this time more thorough investigation into this relationship is needed before we can assign more substantive conclusions or meanings.

Interpretation of ERβ Results

The female ethanol-fed rats' elevated ERβ mRNA expression put their ERβ mRNA levels on par with males'. This corresponds with the ethanol-fed females' more male-like reduced excretion of a water load. This data is consistent with the belief that estrogen acts to inhibits the antidiuretic action of VP (10) and would help explain propensity for water loss during the metestrous phase when estrogen is elevated.

Conclusion

The hypothesis stating that when subjected to chronic alcohol exposure, male and female rats will exhibit different water load handling responses via differences in renal V2R or ER expression was supported by this study. water load excretion and mRNA

expression in male rats remained unaltered by ethanol exposure. In contrast, female rats responded to ethanol exposure with reduced water load excretion and upregulated V2R mRNA in the renal papilla compared to controls. In addition, ethanol-fed females also displayed ERβ upregulation in the renal papilla with respect to control females. Ultimately, these results suggest that unlike male rats, female rats are uniquely able to conserve water and compensate for the dehydrating effects of alcohol by increasing renal responsiveness to VP via upregulating renal V2R. Also, ethanol specifically upregulates the ERβ subtype in the female kidney which may modulate renal sensitivity to VP.

REFERENCES

1. Koeppen Bruce M, S. B. A. (2007) *Renal Physiology*, Mosby and Elsevier
2. Pouzet, B., Serradeil-Le Gal, C., Bouby, N., Maffrand, J. P., Le Fur, G., and Bankir, L. (2001) Selective blockade of vasopressin V2 receptors reveals significant V2-mediated water reabsorption in Brattleboro rats with diabetes insipidus. *Nephrol Dial Transplant* **16**, 725-734
3. Sokol HW, V. H., ed (1982) *The Brattleboro Rat*, The New York Academy of Sciences, New York
4. Rennke Helmut G., D., Bradley M. (2007) *Renal Pathiophysiology, The Essentials*, Lippincott Williams & WIlkins
5. Gulya, K., Dave, J. R., and Hoffman, P. L. (1991) Chronic ethanol ingestion decreases vasopressin mRNA in hypothalamic and extrahypothalamic nuclei of mouse brain. *Brain research* **557**, 129-135
6. Swenson, K. L., and Sladek, C. D. (1997) Gonadal steroid modulation of vasopressin secretion in response to osmotic stimulation. *Endocrinology* **138**, 2089-2097
7. Perucca, J., Bouby, N., Valeix, P., and Bankir, L. (2007) Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. In *American journal of physiology* Vol. 292 pp. R700-705
8. Wang, Y. X., Crofton, J. T., Miller, J., Sigman, C. J., Liu, H., Huber, J. M., Brooks, D. P., and Share, L. (1996) Sex difference in urinary concentrating ability of rats with water deprivation. *The American journal of physiology* **270**, R550-555
9. Slimmer, L. M., and Blair, M. L. (1996) Female reproductive cycle influences plasma volume and protein restitution after hemorrhage in the conscious rat. *The American journal of physiology* **271**, R626-633
10. Wang, Y. X., Crofton, J. T., Liu, H., Sato, K., Brooks, D. P., and Share, L. (1995) Estradiol attenuates the antidiuretic action of vasopressin in ovariectomized rats. *The American journal of physiology* **268**, R951-957
11. CDC (2007) High Blood Pressure Facts and Statistics.
12. CDC (2007) Diabetes Data & Trends. Centers for Disease Control and Prevention
13. Keyes, K. M., Grant, B. F., and Hasin, D. S. (2007) Evidence for a closing gender gap in alcohol use, abuse, and dependence in the United States population. *Drug Alcohol Depend*
14. Robinson, D. L., Brunner, L. J., and Gonzales, R. A. (2002) Effect of gender and estrous cycle on the pharmacokinetics of ethanol in the rat brain. *Alcoholism, clinical and experimental research* **26**, 165-172

15. Alfonso, M., Duran, R., and Marco, J. (1993) Ethanol-induced alterations in gonadotrophins secretion during the estrous cycle of rats. *Alcohol and alcoholism (Oxford, Oxfordshire)* **28**, 667-674
16. Hoffman, P. L., and Tabakoff, B. (1989) Mechanisms of alcohol tolerance. *Alcohol and alcoholism (Oxford, Oxfordshire)* **24**, 251-252
17. Gellai, M., and Valtin, H. (1979) Chronic vascular constrictions and measurements of renal function in conscious rats. *Kidney international* **15**, 419-426
18. Uyehara, C. F., and Gellai, M. (1993) Impairment of renal function precedes establishment of hypertension in spontaneously hypertensive rats. *The American journal of physiology* **265**, R943-950
19. Marcondes, F. K., Bianchi, F. J., and Tanno, A. P. (2002) Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian journal of biology = Revista brasleira de biologia* **62**, 609-614
20. Bevan, D. R. (1978) Osmometry. 1. Terminology and principles of measurement. *Anaesthesia* **33**, 794-800
21. Schmittgen, T. D., Zakrajsek, B. A., Mills, A. G., Gorn, V., Singer, M. J., and Reed, M. W. (2000) Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: comparison of endpoint and real-time methods. *Analytical biochemistry* **285**, 194-204
22. Winer, J., Jung, C. K., Shackel, I., and Williams, P. M. (1999) Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. *Analytical biochemistry* **270**, 41-49
23. Claybaugh, J. R., Sato, A. K., Crosswhite, L. K., and Hassell, L. H. (2000) Effects of time of day, gender, and menstrual cycle phase on the human response to a water load. *American journal of physiology* **279**, R966-973
24. De Vito, E., Guardia, D. C., and Cabrera, R. R. (1989) Cyclical changes in plasma renin during the oestrous cycle in the rat: synchronized effect of oestrogen and progesterone. *The Journal of endocrinology* **121**, 261-267
25. Hartley, D. E., and Forsling, M. L. (2002) Renal response to arginine vasopressin during the oestrous cycle in the rat: comparison of glucose and saline infusion using physiological doses of vasopressin. *Experimental physiology* **87**, 9-15
26. Probst, R. J., Lim, J. M., Bird, D. N., Pole, G. L., Sato, A. K., and Claybaugh, J. R. (2006) Gender differences in the blood volume of conscious Sprague-Dawley rats. *J Am Assoc Lab Anim Sci* **45**, 49-52
27. Stachenfeld, N. S., and Taylor, H. S. (2005) Progesterone increases plasma volume independent of estradiol. *J Appl Physiol* **98**, 1991-1997
28. Canoine, V., Fusani, L., Schlinger, B., and Hau, M. (2007) Low sex steroids, high steroid receptors: Increasing the sensitivity of the nonreproductive brain. *Developmental neurobiology* **67**, 57-67
29. Cheong, B., Muthupillai, R., Rubin, M. F., and Flamm, S. D. (2007) Normal values for renal length and volume as measured by magnetic resonance imaging. *Clin J Am Soc Nephrol* **2**, 38-45

30. Sun, J., Langer, W. J., Devish, K., and Lane, P. H. (2006) Compensatory kidney growth in estrogen receptor-alpha null mice. *Am J Physiol Renal Physiol* **290**, F319-323
31. Taylor, A. H., and Al-Azzawi, F. (2000) Immunolocalisation of oestrogen receptor beta in human tissues. *Journal of molecular endocrinology* **24**, 145-155
32. Suzuki, S., and Handa, R. J. (2004) Regulation of estrogen receptor-beta expression in the female rat hypothalamus: differential effects of dexamethasone and estradiol. *Endocrinology* **145**, 3658-3670
33. Razandi, M., Pedram, A., and Levin, E. R. (2000) Plasma membrane estrogen receptors signal to antiapoptosis in breast cancer. *Molecular endocrinology (Baltimore, Md* **14**, 1434-1447
34. Kalita, K., Szymczak, S., and Kaczmarek, L. (2005) Non-nuclear estrogen receptor beta and alpha in the hippocampus of male and female rats. *Hippocampus* **15**, 404-412
35. Bjurlin, M. A., and Byron, K. L. (2005) Protein-protein interactions in vasopressin signal transduction: new evidence for cytoskeletal dynamics. *The Journal of the American Osteopathic Association* **105**, 24-25
36. Bouley, R., Lin, H. Y., Raychowdhury, M. K., Marshansky, V., Brown, D., and Ausiello, D. A. (2005) Downregulation of the vasopressin type 2 receptor after vasopressin-induced internalization: involvement of a lysosomal degradation pathway. *Am J Physiol Cell Physiol* **288**, C1390-1401

SECURITY AND POLICY REVIEW WORKSHEET- Request for Public Release Clearance

(In Accordance With AFI 35-101, Chapter 15)

(Do not use this worksheet to request clearance of software or web pages.)

SUBMITTING ORGANIZATION *(Office Symbol)* AFIT/CI

THD 80202

A. DOCUMENT TYPE *(Documents must be complete including all figures, charts, photographs and text.)*

☐ ABSTRACT	☐ JOURNAL ARTICLE	☒ THESIS	☐ DISSERTATION
☐ BROCHURES	☐ NEWS RELEASE	☐ SPEECH	☐ OTHER
☐ CD-ROM	☐ PHOTO WITH CAPTIONS	☐ SUCCESS STORY	
☐ DISPLAY/EXHIBIT	☐ POSTER SESSION	☐ TECHNICAL PAPER	
☐ FACT SHEET	☐ PRESENTATION WITH	☐ TECHNICAL REPORT	

B. TITLE OF DOCUMENT: Water Sparing in Chronic Ethanol Exposure is Associated With Elevated Renal Estrogen Receptor Beta & Vasopressin V2 Receptor mRNA in the Female Rat

NO. PAGES: 31

C. AUTHOR(S) NAME AND DUTY TITLE: Maj Odaro J. Huckstep AFIT/CI student

OFFICE SYMBOL: AFIT/CI

D. FORUM *(Public release clearance is not required for material presented in a closed meeting and which will not be made available to the general public on the Internet or in any print or electronic media)*

DOCUMENT WILL BE	NAME OF CONFERENCE	LOCATION	DATE
☐ **PRESENTED ORALLY**			
DOCUMENT WILL BE	**NAME OF PUBLICATION**		**SUBMITTAL DEADLINE**
☐ **DISTRIBUTED IN PRINT**			

HAVE RELATED DOCUMENTS BEEN PREVIOUSLY CLEARED FOR PUBLIC RELEASE

☐ **NO** ☐ **YES** **AFIT CASE NUMBER** _____

NOTE: If document will be released to a medium outside the Department of Defense, the following disclaimer must be added: *The views expressed in this article are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.*

FOR AFIT/PA USE ONLY

SUBMIT TO: Air Force Institute of Technology
Public Affairs (AFIT/PA)
Building 642, Room 2010
2950 P Street
Wright-Patterson AFB OH 45433-7765

FOR MORE INFORMATION

Phone: (937) 255-9354 DSN: 785-9354
FAX: (937) 255-2135 DSN: 785-2135
E-MAIL: afit.pa@afit.edu
WEB: http://www.afit.mil/newsrm01.htm

E. NATIONAL SECURITY AND TECHNOLOGY ISSUES

CHECK YES OR NO *(Provide a brief explanation for all positive responses in the SYNOPSIS SECTION below.)*

☐ YES	☒ NO	DOES THE MATERIAL HAVE THE POTENTIAL TO BECOME AN ITEM OF NATIONAL OR INTERNATIONAL INTEREST.
☐ YES	☒ NO	DOES THE MATERIAL AFFECT NATIONAL SECURITY POLICY OR FOREIGN RELATIONS.
☐ YES	☒ NO	DOES THE MATERIAL CONCERN SUBJECTS OF POTENTIAL CONTROVERSY AMONG DOD COMPONENTS OR WITH OTHER FEDERAL AGENCIES.
☐ YES	☒ NO	DOES THE MATERIAL CONTAIN TECHNICAL DATA DEVELOPED UNDER CONTRACT OR INDEPENDENTLY DEVELOPED AND CONTROLLED BY THE INTERNATIONAL TRAFFIC IN ARMS REGULATIONS (ITAR) THAT MAY BE MILITARILY CRITICAL AND SUBJECT TO LIMITED DISTRIBUTION, BUT ON WHICH A DISTRIBUTION DETERMINATION HAS NOT BEEN MADE.
☐ YES	☒ NO	DOES THE MATERIAL CONTAIN INFORMATION ON NEW WEAPONS OR WEAPON SYSTEMS, SIGNIFICANT MODIFICATIONS OR IMPROVEMENTS TO EXISTING WEAPONS OR WEAPON SYSTEMS, EQUIPMENT OR TECHNIQUES.
☐ YES	☒ NO	DOES THE MATERIAL CONTAIN INFORMATION ON NATIONAL COMMAND AUTHORITIES; COMMAND, CONTROL, COMMUNICATIONS, COMPUTERS AND INTELLIGENCE; INFORMATION WARFARE; OR COMPUTER SECURITY.
☐ YES	☒ NO	DOES THE MATERIAL CONTAIN INFORMATION ON MILITARY ACTIVITIES OR APPLICATIONS IN SPACE, NUCLEAR WEAPONS, INCLUDING WEAPON-EFFECTS RESEARCH; CHEMICAL AND BIOLOGICAL WARFARE ISSUES; BIOLOGICAL AND TOXIN RESEARCH, HIGH-ENERGY LASERS AND PARTICLE BEAM TECHNOLOGY; ARMS CONTROL TREATY

F. SNYNOPSIS *(Provide a brief description of the system, process, or technology, including its state of development and whether the application is military, commercial, or dual-use).* EXAMPLE: This is new concept of applying current development for high-powered lasers, specifically for military applications.

Dual-use, this research advances our knowledge of the effects of chronic alcohol intake. Specifically targeting gender-differences in body fluid (water) management.

G. REFERENCES *(Check all that apply)*

☒ ALL REFERENCES ARE UNCLASSIFIED, UNLIMITED, AND ARE AVAILABLE TO THE PUBLIC

☐ REFERENCE # ARE SUBJECT TO DISTRIBUTION LIMITATION. NO LIMITED INFORMATION FROM THESE REFERENCES IS INCLUDED IN THE DOCUMENT

☐ NO REFERENCES ARE INCLUDED IN THIS DOCUMENT

H. **RELEASE AUTHORITY AND MANAGEMENT RESPONSIBILITY FOR JOINT EFFORTS**

IS THIS INFORMATION CO-AUTHORED WITH SOMEONE FROM AN ORGANIZATION OUTSIDE OF AFIT.

☐ YES ☒ NO

DOES YOUR ORGANIZATION HAVE THE AUTHORITY TO RELEASE THE INFORMATION

☒ YES ☐ NO

IF WORK IS SPONSORED BY ANOTHER ORGANIZATION, DO YOU HAVE THE AUTHORITY TO RELEASE ALL THE INFORMATION

☒ YES ☐ NO

IF NOT, WHAT ORGANIZATION SHOULD COORDINATE ON THE REQUEST

Tripler Army Medical Center PA for Dept of Clinical Investigation

ORGANIZATION	PHONE
TAMC/PA	808 433 5785

NOTE: If the organization is a DOD organization, AFIT/PA will coordinate release approval with that organization's PA office

I.	AUTHOR/TECHNICAL REVIEW CERTIFICATION

AUTHOR

I certify the information contained in the attached document is technically accurate and does not disclose classified, sensitive, or militarily critical technology and does not violate proprietary rights or copyright restrictions. All security and technology issues listed above have been considered and any applicable security classification guides have been reviewed.

SIGNATURE	DATE
	10 Jan 2008
AUTHOR(S) NAME (Print)	TELEPHONE
Odaro John Huckstep Maj USAF	808-203-8163
OFFICE SYMBOL/ORGANIZATION	E-MAIL ADDRESS
AFIT/CI	odaro@hawaii.edu

TECHNICAL REVIEWER CERTIFICATION (Faculty Advisor)

I certify the information contained in the attached document is technically accurate and does not disclose classified, sensitive, or militarily critical technology and does not violate proprietary rights or copyright restrictions. All security and technology issues listed above have been considered and any applicable security classification guides have been reviewed.

SIGNATURE	DATE
	24 Jan 2008
TECHNICAL REVIEWER'S NAME (Print)	TELEPHONE
CATHERINE F. T. UYEHARA	808 433 7163
OFFICE SYMBOL/ORGANIZATION	E-MAIL ADDRESS
MCHK-CI	catherine.uyehara@amedd.army.mil

DEPARTMENT HEAD *(or equivalent)*/PROGRAM MANAGER CERTIFICATION

The attached material submitted for public release has been properly staffed and reviewed by this division/organization. I support the recommendation of the Author and Technical Reviewer for public release.

SIGNATURE	DATE
DUSTI R. JAYNE, Capt, USAF Chief, Faculty Pipeline Programs Air Force Institute of Technology	FEB 13 2008
NAME *(Print)* Scott Lozanoff	TELEPHONE 785 2259
OFFICE SYMBOL/ORGANIZATION	

AFIT PUBLIC AFFAIRS

THE ATTACHED MATERIAL IS/IS NOT CLEARED FOR PUBLIC RELEASE

SIGNATURE OF SECURITY AND POLICY REVIEW OFFICER	DATE
	25 Feb 08

J. REMARKS *(please reference the specific section)*

GENERAL INSTRUCTIONS

The information provided on this worksheet will be used by the Air Force Institute of Technology Public Affairs Directorate to process requests for Security and Policy Review in accordance with Air Force Instruction 35-101, Chapter 15. Do not use it to request clearance of software source code or web pages.

To expedite processing of your request, please print legibly and complete all sections of the worksheet. Space is provided in Section K for additional remarks. If used, please specify the section commented on.

Please submit one copy of both this worksheet and the document. We will contact you if additional copies are required. Documents may be submitted in print or digital format (CD-ROM, DVD, or Zip Disk) or VHS videotape.

SECTION INSTRUCTIONS

SECTION A:	**DOCUMENT TYPE** - Identifies the intended presentation format for the information. Place a check in the appropriate box.
SECTION B:	**TITLE OF DOCUMENT** - Complete document title *(Explain abbreviations/acronyms in Section J)*
SECTION C:	**AUTHOR(S) NAME, DUTY TITLE, OFFICE SYMBOL** - Author's first and last name, position or duty title and complete office symbol (e.g., AFIT/PA)
SECTION D:	**FORUM** - Indicate the method that will be used to present the information. If for presentation at a conference or meeting, provide the full name of the conference or meeting. Provide the location (city, state) and date of the event. If for print media, provide the name of the publication and submission deadline. If related documents have been previously cleared for public release, provide the AFIT case number. *(An example of a document that has been previously cleared is as follows: If a thesis has been cleared and you are extracting information from that thesis for another article you have written. Please include the case number of the thesis.)*
SECTION E:	**NATIONAL SECURITY AND TECHNOLOGY ISSUES** - Answer each question and explain positive responses in Section F or J.
SECTION F:	**SYNOPSIS** - Provide a brief description of the system, process, or technology, including its state of development and whether its application is military, commercial, or dual-use.
SECTION G:	**REFERENCES** - Check all that apply.
SECTION H:	**JOINT AND INTERNATIONAL EFFORTS** - Indicate whether or not the information resulted from any effort with an external organization or person or with another DOD or U.S. government agency and specify which organization has release authority and program management responsibility.
SECTION I:	**TECHNICAL REVIEW AND CERTIFICATION** - The Author and Technical Reviewer (faculty advisor) must certify that the information complies with the statement. (The technical reviewer section applies only when students are submitting documents.) A department head or deputy (or equivalent) or program manager must sign in the second block affirming support for public release.
SECTION J:	**REMARKS**: Use when additional space is needed to explain a response. Please indicate the section to which the explanation applies. Explain any abbreviations or acronyms.

NOTE:

ALL PERSONAL INFORMATION IN THE FOLLOWING CATEGORIES ABOUT U.S. CITIZENS, DOD EMPLOYEES AND MILITARY PERSONNEL SHOULD NOT BE INCLUDED IN THESES, DISSERTATIONS OR OTHER DOCUMENTS THAT WILL BE POSTED ON THE WORLD-WIDE WEB:

1) SOCIAL SECURITY ACCOUNT NUMBERS
2) DATES OF BIRTH
3) HOME ADDRESSES
4) TELEPHONE NUMBERS OTHER THAN NUMBERS OF DUTY OFFICERS WHICH ARE APPROPRIATELY MADE AVAILABLE TO THE GENERAL PUBLIC
5) NAMES, LOCATIONS AND ANY OTHER IDENTIFYING INFORMATION ABOUT FAMILY MEMBERS OF DOD EMPLOYEES AND MILITARY PERSONNEL